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Determining
COMMON ROOT ROT POTENTIAL of PEA FIELDS

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Summary

Fields having high infestations of *Aphanomyces euteiches,* the fungus causing common rot of pea, can be distinguished from root-rot-free fields prior to planting by a new and simple greenhouse test. A representative soil sample from the field is planted with peas in 6-inch pots. The greenhouse is kept at 75° F and pots are watered heavily daily for two weeks after the 2-leaf stage to insure rapid infection of plants in infested soils. After 28–33 days a disease index is calculated for each soil sample. A high index indicates that the field is unsafe for planting peas; a low index shows that the field will have little or no root rot. The technique was applied to a test of 61, 52, and 42 Wisconsin fields in 1955, 1956, and 1957, respectively. There was a highly significant positive correlation of predicted root rot with root rot in the field, and a highly significant inverse correlation of the amount of root rot and the pea yields.

Determining the Common Root Rot Potential of Pea Fields[1]

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THE most destructive disease of canning peas in Wisconsin is common root rot caused by the fungus *Aphanomyces euteiches*. Attempts to control this disease by using crop rotations or by breeding resistant varieties have not been successful, so another approach is obviously in order. Since damage is of significance primarily in fields having a moderate to high infestation of the causal fungus, economic losses due to common root rot can be greatly reduced by withholding peas from all land known to have such infestations.

A greenhouse technique for estimating the degree of infestation of fields prior to planting has been developed by the University of Minnesota and used with good success by at least two large commercial canners. A representative soil sample is collected from the field and planted with peas in the greenhouse. The condition of the roots at the blossoming stage indicates the amount of root rot that could develop in the field. Fields found to have more than a certain level of infestation are not recommended for planting to peas.

An improved technique for determining root rot potential has been developed by the writers. This bulletin describes this new technique and presents the results of three years' evaluation of this test.

Methods and Materials

Collecting Soil Samples

The field should be sampled the fall before peas are to be planted. The person taking the sample walks across the field and back on a zig-zag course. Every 55 to 75 paces (not steps), a trowelful of soil (about one pint) is taken to a 6-inch depth and placed in a 15-pound polyethylene bag (10″ × 8″ × 24″ turkey freezer bag) carried, for convenience, in a 12-quart pail. Usually one bag is inserted within another to prevent breakage. Trowelfuls of soil are taken from 10 locations in fields 10 acres or smaller and from one location per acre in larger fields. All the trowelfuls from a field are placed together in one double bag and constitute the sample. At least 5 quarts of soil are needed for the test. To obtain a sample that is representative of the field, trowelfuls should be taken from all parts of the field and from areas having different drainage conditions, soil types, and elevations in the same proportions as their relation to the total area of the field. SMALL LOW SPOTS SHOULD

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NOT BE SAMPLED. If a part of the field has recently been in peas, this area should be sampled and tested separately. Very large fields should be divided into 2 or more areas of equal size and each area tested separately.

The canning company or other sampling agency should assign a code number to the field and keep a record of the name of the farm, location of the field, name of the sampler, date of sampling, size of the field, known cropping history with regard to peas, and results of the test. Store samples in a clean dry place.

Testing Soil Samples

Six-inch earthenware pots are washed and sterilized. The hole in the bottom of each pot is covered with a large piece of pot label to limit drainage. The soil sample is placed on clean newspaper on a potting table, all lumps are broken up, and the sample is thoroughly mixed by hand. Three pots are filled to within two inches of the top with the mixed soil. Eleven fungicide-treated seeds of Pride variety pea are placed on the soil in each pot and covered with an additional inch of soil. The pots are labeled and randomized on a greenhouse bench.

Fig. 1.—Check plants in A. euteiches-*free (left) and* A. euteiches-*infested (right) soils at time of grading for greenhouse common root rot disease index.*

Every time a test is begun with one or more samples, check or control plantings must also be made. Two controls are used—1) a known highly infested soil, and 2) a known root-rot-free soil. Three 6-inch pots of each of these controls are recommended.

The improved method is based upon providing a controlled greenhouse environment which will insure rapid development of this disease when *A. euteiches* is present in the soil. Therefore, proper management of temperature and soil moisture are essential to the success of the test. Greenhouse temperature is kept between 75° and 83° F, day and night, throughout the test. Constant 75° F is most desirable. The soil is kept at a normal, moderate moisture level during seed germination and emergence by watering lightly as needed. Emergence from soils that crust is aided by slight daily watering. Beginning when the first two seedling leaves are fully expanded, 9 to 11 days after planting, the soil is held at or near saturation by heavy daily watering for 12 to 15 days. Then soils are kept at normal moisture until final readings are made. It is advisable to use warm water near 75° F.

Determining Greenhouse Disease Index

Root rot readings are made when all plants in the heavily infested check soil are dead, usually 28 to 33 days after planting. The appearance of check plants at this stage is illustrated in Figure 1. Samples potted the same day are evaluated at one time. The soil ball is removed from the pot, and roots are carefully removed and freed of soil by

Fig. 2.—Pea plants from greenhouse assay for root-rot potential showing, from left to right, appearance of roots in disease classes 0, 1, 2, 3, and 4, respectively.

washing under running water. Each root is assigned to one of the following arbitrary disease classes:

0—no disease, roots free of symptoms

1—a few water soaked, light brown areas on roots

2—water soaked, light brown areas confluent and more extensive but not involving the entire root system, tissue firm

3—water soaking and browning involving all roots and epicotyl (stem above seed piece), tissue soft but not collapsed, epicotyl not markedly shriveled

4—water soaking, browning, and decay involving all roots and epicotyl, cortex easily sloughed off, epicotyl shriveled or rotted

Dead plants are put in class 4. The disease classes are illustrated in Figure 2. It is important to note that stem and leaf symptoms are not considered. Only root symptoms attributable to *A. euteiches* are graded so that such symptoms as reddening of the stele, black discoloration of the epicotyl, and firm brown discoloration at the attachment of the cotyledons are not considered.

A disease index is calculated for each pot using the following formula:

$$\text{Disease index} = \frac{\text{sum of}\left(\text{disease class} \times \text{number of plants in that class}\right) \times 100}{\text{total number of plants} \times 4}$$

Sample calculation:

						sum
(1) disease class	0	1	2	3	4	
(2) number of plants in that class	0	0	6	4	1	11
(1) × (2)	0	0	12	12	4	28

$$\text{disease index} = \frac{28 \times 100}{11 \times 4} = 64$$

A disease index of 0 means that all the plants are healthy, 100 means that all are severely infected, and intermediate values represent intermediate infection. The average of the 3 replicate pot indices is designated the greenhouse index of the soil sample. The greenhouse index is taken as a measure of the root-rot potential of the field.

Deciding Whether or Not to Plant Peas

The results of three years' experience shows that fields with a greenhouse index of 0 to 50 can safely be planted with peas, fields with an index of 51 to 69 are of questionable safety, and fields testing 70 to 100 are definitely dangerous and should not be planted to peas. Peas should be withheld from fields in the 51 to 69 range whenever possible, particularly if they have been grown there within the previous 5 or 6 years. In any event, only early maturing varieties should be planted in such fields.

Experimental Studies

The relation of the greenhouse test for root-rot potential, the actual amount of root rot in commercial pea fields, and the yields of these fields in Wisconsin was studied by the authors in 1955, 1956, and 1957.

Soil samples were taken before planting from commercial fields in the vicinity of Janesville, Columbus, Brandon, Oakfield, Green Bay, Marshfield, and New Richmond in 1955 and 1956, Holmen in 1955, Belgium in 1956, and Janesville, Columbus, Brandon, Oakfield, and Waunakee in 1957. Six to 9 fields were sampled in each area each year. The greenhouse test described above was run on each sample.

The fields were revisited just before harvest and graded for the actual amount of root rot in the field. Roots were dug up with a trowel at intervals of 15 to 25 paces and carefully examined. On the basis of this, an estimate was made of the percentage of the total area of the field having roots falling into the classes: none, slight, moderate, and severe root rot. Weighted values of 0, 1, 2, and 3, respectively, were assigned to the classes, and a field disease index was calculated by the formula:

$$\text{Field index} = \frac{\text{sum of} \left(\begin{matrix} \text{per cent of total} \\ \text{area in class} \end{matrix} \times \begin{matrix} \text{class} \\ \text{value} \end{matrix} \right)}{3}$$

The field index can be compared directly with the greenhouse index, a field index of 0 indicating that there is no root rot in the field, 100 indicating that 100 per cent of the field had severe root rot, and intermediate values indicating intermediate amounts of root rot.

Yield and tenderometer data from the fields were supplied by the canning companies concerned. Because fields were harvested at different stages of maturity, and because weight of peas increases with increase in maturity, all yields were adjusted to the theoretical yields at a standard maturity represented by a tenderometer reading of 100. The following method of calculation, based on data of Hagedorn, Holm, and Torrie,[3] was used:

$$\text{Yield at tenderometer 100} = \text{actual yield} - \left(\begin{matrix} \text{tenderometer} \\ \text{reading} \end{matrix} - 100 \right) \times 28.7$$

The adjusted yield is lower than the actual yield if the tenderometer is greater than 100 and higher if the tenderometer is less than 100.

The coefficients of correlation between greenhouse indices, field indices, and yields were calculated. A coefficient of + 1 indicates a perfect, positive relationship between the two variables compared; − 1 indicates a perfect, inverse relationship; 0 indicates complete absence of relationship; and intermediate values indicate intermediate degrees of correlation. In the tables, one asterisk indicates significance at the 5 per cent level; two asterisks indicate significance at the one per cent level.

[3] Hagedorn, D. J., L. G. Holm, and J. H. Torrie. Yield-quality relationships as influenced by maturity of canning peas. Wis. Res. Bul. 187. August, 1955.

Experimental Results

Greenhouse Index, Field Root Rot and Yield Relationships

In all three years there was a striking correlation between the greenhouse index and the amount of root rot in the field, and there was a decrease in yield with increase in root rot. The 1957 results given in Table 1 are typical of the data. Correlation coefficients for all years are given in Table 2. The interpretation of these results is given in the following paragraphs.

Table 1.—Greenhouse and field common root rot disease indices and yields of pea fields in 1957

Field code number	County	Common Root Rot Disease Index[a]		Yield[b] lb./A
		Greenhouse	Field	
120	Rock	0	0	2785
118	Rock	1	0	2807
124	Rock	2	0	2621
117	Rock	3	3	passed
125	Rock	3	0	2043
150	Dane	3	2	3379
148	Dane	4	0	1358
142	Dane	22	2	1757
126	Rock	32	0	2043
149	Dane	32	22	1485
136	Fond du Lac	45	3	
146	Dane	45	0	3395
119	Rock	47	0	4198
155	Fond du Lac	51	0	2688
134	Fond du Lac	56	48	1955
145	Dane	64	40	1621
144	Dane	66	22	1621
121	Rock	73	12	passed
156	Fond du Lac	77	33	2002
129	Fond du Lac	78	7	1993
147	Dane	80	75	511
127	Fond du Lac	85	10	1993
130	Fond du Lac	88	3	1993
154	Fond du Lac	91	9	2575
122	Rock	92	38	960
157	Fond du Lac	92	32	1581
131	Fond du Lac	93	32	2033
152	Dane	95	90	0
132	Fond du Lac	96	60	2033
135	Fond du Lac	96	45	1955
158	Fond du Lac	97	64	1830
123	Rock	98	38	960
140	Fond du Lac	98	62	2179
141	Columbia	98	53	1138
151	Dane	98	90	0
133	Fond du Lac	99	68	1708
138	Fond du Lac	99	50	3106
139	Fond du Lac	99	50	1397
128	Fond du Lac	100	17	1993
137	Fond du Lac	100	58	1279
143	Dane	100	67	1559
153	Dane	100	100	0

[a] 0 = no disease, 100 = very severe disease.
[b] Yields are adjusted to theoretical yield at tenderometer 100.

The highly significant positive correlation of the greenhouse index with the field index all three years indicates that the greenhouse test predicts with reasonable accuracy the amount of common root rot which can develop in the field. Whenever the sample has a low greenhouse index, the field also has little or no root rot. Thus one can plant peas in any field having a low greenhouse index with the assurance that there will not be a root rot problem. A severe outbreak of root rot in a field was always anticipated by a high greenhouse index for that field. Therefore, the test detects all highly infested fields. One would not be deceived into thinking that the land was free of *A. euteiches* and later be confronted with extensive depredations by that fungus.

There were some instances in which a sample had a high greenhouse index, yet little or no root rot developed in the field. In some of these cases the actual root rot potential of the field was undoubtedly high, as indicated by the high

Table 2.—Correlation coefficients for pea root rot disease greenhouse indices, field indices, and yields adjusted to tenderometer 100 in 1955, 1956, and 1957

	Correlation coefficients 1955	
	Field index	Yield
Greenhouse index	0.56**	—0.18
Field index		—0.37**
	1956	
	Field index	Yield
Greenhouse index	0.74**	—0.24
Field index		—0.35*
	1957	
	Field index	Yield
Greenhouse index	0.71**	—0.45**
Field index		—0.70**

greenhouse index, but the local weather was unfavorable for the disease that particular season, and the peas escaped serious damage. In other instances the true root rot potential of the field may have been lower than was implied by the greenhouse index. Experiments have indicated this would happen if in taking the sample one or more trowelfuls of soil were drawn from small, heavily infested pockets in a generally uninfested field. The disease index of a composite sample made up of subsamples differing in level of *Aphanomyces* infestation tends to be higher than the average of the disease indices of each of the subsamples. For this reason, it is recommended that small low spots be avoided in taking the samples.

That yield was inversely related to the amount of root rot in the field was shown by the coefficients of correlation of the field index with yield. These correlations were significant at the one per cent level in 1955 and 1957, and at the 5 per cent level in 1956. Root rot was especially important in reducing yield in 1957. The square of the correlation coefficient for 1957 is 0.49. This figure indicates that of all the factors responsible for variation in yield, variations in root rot accounted for about 49 per cent of the total variation in yield. There was about twice the normal amount of rainfall in May and June, 1957, and it was well distributed throughout the growing season. This favored root rot in fields with high root-rot potentials and promoted high yields in root-rot-free fields.

There was an inverse relationship between the root-rot potential of a field, as estimated by the greenhouse index, and the yield of peas. The correlation was highly significant in 1957. In that year variation in root-rot potential accounted for about 25 per cent of the variation in yield.

Several fields were abandoned because of severe root rot. In many others the yields were so low that there was a serious loss on the investment in material and labor.

Several factors may reduce the correlations of greenhouse index, field index, and yield. Low rainfall suppresses root rot development in heavily infested fields and depresses the yield of uninfested fields. High rainfall at the end of the season is conducive to root rot development without corresponding yield reduction. Inclusion of heavily infested subsamples from a predominantly uninfested or lightly infested field biases the greenhouse index upward. Yield is influenced by many factors among which are tillage, seeding rate, plant stand, weed competition, soil fertility, pea variety, and diseases other than root rot. That the correlations of greenhouse index, field index, and yield were significant in this study, in spite of these other factors, demonstrates the ability of the test to estimate root-rot potential and emphasizes the importance of root rot in reducing yield.

Yields in Different Years

The three years 1955–57 were compared with respect to yields of fields having slight, moderate, and severe root rot. Fields indexing between 0 and 25 were considered to have slight root rot; 26 to 50 was considered moderate, and 51 to 100 was considered severe. Yields of all fields in each of the 3 classes were averaged for each year, and the significance of the differences was determined by the *t* test. The results are given in Table 3.

Table 3.—Average yields of fields having different amounts of common pea root rot as indicated by the field disease indices

Amount of root rot	Field index range	Average yield, lbs./A, at tenderometer 100 for the year		
		1955	1956	1957
Slight	0– 25	2053	2183	2371
Moderate	26– 50	1910	2351	1633
Severe	51–100	1692	1738	1530
	51–100[a]	1493	1564	1112

[a]Includes fields not harvested because of root rot. These fields were given a 0 yield and were not included in calculations of *t*.

Fields with slight root rot had a higher average yield than fields with severe root rot all three years. The difference was significant at the one per cent level in 1957 and at the 10 per cent level in 1955. In 1955 and 1957 fields with slight root rot outyielded fields with moderate root rot. The average yield with moderate root rot was consistently greater than with severe root rot, significantly so in 1956.

Fields with little or no root rot tended to yield more in 1957 than in 1955 or 1956 as was to be expected in this year of high rainfall. In contrast to this, fields with moderate or severe disease tended to yield less in 1957 than comparable fields in years of more normal rainfall, even when only harvested fields were considered. This finding is contrary to present belief which holds that fields with severe root rot produce more peas in wet years than do similarly affected fields in normal years. Extensive infection of the root system early in the life of the plant is known to reduce final growth more than equivalent infection of larger plants. In heavily infested fields the increased likelihood of early spread of the disease in a wet year may account for the apparently greater loss.

Disease Index and *Aphanomyces euteiches*

Although a number of fungi are known to cause rotting and browning of pea roots, *Aphanomyces euteiches* is generally said to be the most widespread and destructive pea root pathogen in Wisconsin. A survey was made of the relation of *A. euteiches* to various levels of the greenhouse disease index. Roots were saved from the greenhouse tests of samples from 45 fields in 1956 and 42 fields in 1957. Crushed mounts of rotted portions were examined microscopically for characteristic *A. euteiches* oospores. *A. euteiches* was not found in roots grown in soils with an average greenhouse index less than 49. It was seen in roots from some soils indexing from 49 to 55, and in roots from all but 2 soils indexing 56 to 100. Other parasitic fungi were much less frequently observed. Thus it is clear that *A. euteiches* is the major pea root rot organism in Wisconsin and that the greenhouse indexing procedure is a reliable means of detecting its presence in a field.

Early Varieties vs. Late Varieties

Some canners prefer to plant early maturing varieties on land with a root rot problem in the belief that they are not as severely affected by root rot as late varieties. Data supporting this viewpoint were obtained in a study of 25 fields of early varieties and 30 of late varieties in 1955. With the early varieties the average yield of fields having a field disease index between 0 and 50, i. e. low root rot, was 1707 lbs. per acre, and of fields with an index between 51 and 100, i. e. high root rot, was 1402 lbs. per acre, a reduction of 305 lbs. per acre or 18 per cent of the yield. The late varieties averaged 2125 lbs.

per acre in fields indexed 0 to 50, and only 1595 lbs. in fields indexed 51 to 100, a reduction of 530 lbs. or 25 per cent. Thus in stricken fields late varieties produced more peas than early varieties, but the proportion of peas lost was less when early peas were grown.

Development of the Greenhouse Test

The greenhouse test developed and in use in Minnesota takes up to 60 days for completion, monopolizing valuable greenhouse space for a considerable time. In the present study a method that will give accurate, reproducible results in 28 to 33 days was evolved by systematically determining and applying the environmental conditions most favorable for disease development in *A. euteiches*-infested soil. Different pea varieties and sizes of pots were evaluated for their suitability in the test.

Soil samples were collected and mixed as previously described. Each sample was then divided among the various treatments in the experiment. All tests were replicated three times. Treatments were compared by the analysis of variance after the disease indices were converted to the appropriate angles. The results are given in the following sections.

Effect of Watering. Watering practice affects both the speed and the end result of the test.

Light watering and heavy watering were compared in one experiment. Samples from 22 fields were tested at 80° F and from 20 others at 69°. Three pots of each sample were watered very lightly every third day and three others were watered heavily daily for 2 weeks after the 2-leaf stage. Disease indices were determined when plants in heavily watered infested check soil were dead.

The results at both temperatures were similar. Indices of heavily watered samples ranged from 1 to 100 but most were above 50. *A. euteiches* was found in roots from all soils indexing above 50, showing that most of the soils in the test were infested. The fungus was not found when the index was below 50. The roots of lightly watered plants grown in these same infested soils were almost always white and free of disease. The disease indices of heavily watered soils showed a highly significant correlation with the corresponding field indices. There was no correlation of the indices from light watering with indices of the heavily watered treatment or with field indices.

Obviously, in the greenhouse test watering must be heavy enough for disease to develop or the results are meaningless.

In another experiment, heavy watering was compared with moderate watering. Ten soils were tested at 75° F. One group of pots was watered heavily daily from the 10th to the 30th days after planting. A duplicate group received enough water every second or third day to give optimal plant growth. Indices were determined when root rot was fully developed in infested check soils. Heavily watered plants could be indexed 33 days after planting, while normally watered plants were not ready to be indexed until the 51st day. There was no substantial difference in indices of heavily infested samples given either treatment. The indices of uninfested soils were somewhat higher under moderate watering. Thus the effect of heavy watering was to shorten the test and accentuate the difference between uninfested and heavily infested soils.

Effect of Temperature. Temperature exerts a strong influence on the rapidity

of the test but does not affect the outcome. Trials were conducted in greenhouse rooms kept within 2° F of 61°, 68°, 75°, and 83° F. Pots were watered heavily daily after the 2-leaf stage, and data were taken when plants in infested check soil were dead. At 83° the test was completed in about 24 days, at 75° in 24 to 27 days. At 68° the test took 34 days in the winter, but in spring when plant growth was more vigorous it took 51 days. Plants in all soils matured and produced peas in 75 days at 61° F, but those in infested soil were stunted. Within the temperature range tested the final disease index of any particular soil was quite constant. The results of one trial are given in Table 4.

Table 4.—Greenhouse disease indices of soils tested for pea common root rot potential at different greenhouse temperatures

Field code number	Greenhouse disease indices		
	83°F (24 days)[a]	75°F (27 days)	68°F (51 days)
U	42	5	18
52	15	4	0
24	14	12	38
80	100	100	87
82	98	100	90
13	92	72	86

[a]Number of days from planting until data were taken when infested checks were at equivalent stage of death.

In other experiments, tests made at 75° to 80° were finished in 28 to 33 days, while tests at 68° to 71° took 45 to 55 days. Accordingly, 75° F was selected as the most favorable temperature for the assay.

Effect of Variety. New Era was compared with Pride in a test of 30 soil samples. There was a close agreement of the indices. There were highly significant correlations between the greenhouse indices of the two varieties, and between the greenhouse index of each variety and the field indices. Identical results were obtained in tests with Perfected Wales. Thus New Era and Perfected Wales could be used as test varieties in place of Pride.

Effect of Using 4-Inch Pots. With a view toward increasing the number of samples which could be assayed in a limited amount of space, 4-inch earthenware pots were compared with 6-inch pots in trials with 42 samples. Six seeds were planted per 4-inch pot. There was good agreement of the indices obtained with the two pot sizes, and they were not statistically different. The indices of uninfested or slightly infested soils tended to be slightly higher, and of heavily infested soils to be slightly lower in the 4-inch pots. Three infested samples had unaccountable low indices in the 4-inch pots. The correlations between the two containers and between each container and the field indices were highly significant. It was concluded that 4-inch pots can be substituted for the larger ones if greenhouse space and labor must be conserved but that 6-inch pots seem to give more dependable results.

Importance of These Studies

These studies have shown that fields hazardous for pea production because of heavy infestation with *A. euteiches* can be distinguished from uninfested or slightly infested fields. The yields of heavily infested fields average less than those of uninfested fields. Canning companies can avoid unnecessary loss by routinely testing all fields of unknown root-rot potential and withholding from production fields shown to have dangerous infestation. At the present time this provides the only economical, dependable control of common root rot of peas.